Prevalence of *GCK* mutations in individuals screened for fasting hyperglycaemia

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Abbreviations

DHPLC	denaturing high performance liquid chromatography
EDIT	Early Intervention Diabetes Trial
FHS	Fasting Hyperglycaemia Study
GCK-MODY	glucokinase MODY
2HPGI	2-hour post-challenge plasma glucose increment

To the Editor: Glucokinase is a key enzyme that regulates insulin secretion by changing beta cell glucose phosphorylation rates over the range of physiological glucose concentrations [1]. Inactivating mutations in the gene encoding glucokinase

(*GCK*), as seen in glucokinase MODY (GCK-MODY), shift the threshold value for glucose-stimulated insulin secretion from ~5 to ~7 mmol/l, resulting in elevated fasting plasma glucose (FPG) levels (5.5–8.0 mmol/l) but normal (typically <4.6 mmol/l) 2 h plasma glucose increments (2HPGI) after a 75 g OGTT [2].

Screening for elevated FPG levels to detect undiagnosed type 2 diabetes mellitus and prediabetic states (either impaired fasting glucose or impaired glucose tolerance) is increasingly advocated but could identify a disproportional number of individuals with ‘fixed’ GCK-MODY glucose sensing defects. The prevalence of GCK-MODY is unknown, but individuals with this disease would not normally require glucose-lowering therapy or be at increased risk of type 2 diabetes. The degree to which individuals with GCK-MODY can be distinguished from those with type 2 diabetes or prediabetic states, has not been established. The aim of the present study was to determine the prevalence of *GCK* mutations in individuals screened for fasting hyperglycaemia who were thought to be at increased risk of developing type 2 diabetes, and to establish whether mutation carriers could be distinguished from non-mutation carriers based on clinical criteria alone.

We examined baseline data from the Fasting Hyperglycaemia Study (FHS) [3] and Early Diabetes Intervention Trial (EDIT) [4] to estimate the prevalence of GCK-MODY in individuals with fasting hyperglycaemia (5.5–7.7 mmol/l) on two consecutive occasions, 2 weeks apart. In total, 798 self-referred individuals, aged 30–70 years, who were thought to be at increased diabetes risk were recruited in three FHS and nine EDIT UK clinical centres. Increased risk was defined as the presence of one or more of the following: first-degree relatives with type 2 diabetes; overweight; a history of hyperglycaemia or glycosuria; previous gestational diabetes. All participants gave written informed consent. Participants were excluded if they had known diabetes, were pregnant or possibly could become pregnant, had severe or life-threatening disease, had an uncorrected endocrine

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disturbance, were treated with drugs that affected glucose metabolism, had impaired renal function, uncontrolled hypertension, chronic illness, hepatic insufficiency or alcoholism, or had a BMI >40 kg/m². After recruitment, all participants underwent two 75 g oral glucose tolerance tests, which were conducted 2 weeks apart.

The entire coding region and intron–exon boundaries of *GCK* were amplified and the amplicons were subjected to denaturing high-performance liquid chromatography (DHPLC) analysis. Primers and DHPLC conditions are given in Electronic supplementary material (ESM) Table 1. Abnormal chromatogram samples were sequenced bidirectionally on an ABI3700 sequencer (Applied Biosystems, Warrington, Cheshire, UK) using M13 tailed primers. Sequences were compared with the published sequence (NM_000162.2) using Mutation Surveyor v.3.01 (Biogene, Cambridge, UK) to determine the sequence of the hetero-

duplex. Mutations were confirmed by re-amplifying a freshly diluted sample of DNA.

DHPLC assay sensitivity was determined using DNA from 58 patients with different known *GCK* mutations distributed across the gene. Fifty-seven of 58 mutations (98%) were detected. The missed mutation was located in exon 3 and the sequence was queried but not identified as a definite mutation. Since no abnormal chromatograms were detected for this exon, to ensure that no mutations were missed, exon 3 was sequenced in one direction for all samples.

DNA samples were available from 658 participants (529 FHS, 129 EDIT; mean [±SD] age 52.5±9.8 years; FPG 5.9±0.6 mmol/l; 2HPGI 2.6±2.4 mmol/l; BMI 28.5±4.6 kg/m²) (Table 1). DHPLC analysis of ten islet *GCK* exons identified a total of 209 (2%) abnormal chromatograms out of 10,528. Sequencing of these samples in both

Table 1 Clinical characteristics of FHS and EDIT patients with and without *GCK* mutations

Patient	Enrolment data				Post-enrolment data					
	Age (years)	BMI (kg/m ²)	Ethnic group ^a	Sex ^b	Mean FPG (mmol/l)	2 h glucose increment (mmol/l)	2 h glucose level (mmol/l)	HbA _{1c} (%)	Beta cell function (HOMA %B)	Insulin sensitivity (HOMA %S)
<i>GCK</i> mutation-negative participants (<i>n</i> =653)	52.5±9.8	28.5±4.6	607/7/38/1	330 (50.5%)	6.0±0.5	2.6±2.4	8.6±2.8	5.6±0.6	86 (69, 105)	70 (50, 97)
<i>GCK</i> mutation participants (<i>n</i> =5)	46.5±6.6	30.5±6.0	4/0/1	4 (80%)	6.2±0.6	4.4±2.2	10.6±3.2	5.9±0.3	63 (51, 150)	61 (21, 152)
<i>GCK</i> mutation vs no mutation <i>p</i> value	0.16	0.33		0.37	0.35	0.094	0.098	0.24	0.19	0.78
Individual <i>GCK</i> mutation data ^d										
XJ05356 R369C	45.2	24.7	Indian Asian	M	6.7	6.7	13.8	6.0	59.05	70.30
XJ05070 G175A	57.0	37.8	White Europid	F	6.0	3.9	9.5	6.0	235.95	20.55
XJ05108 V455E	47.5	24.1	White Europid	F	5.7	2.2	7.5	5.8	54.55	191.30
XJ05351 L134P	43.4	33.9	White Europid	F	7.1	6.8	14.0	6.3	70.60	51.50
XJ05049 R186X	39.2	32.2	White Europid	F	5.8	2.5	7.9	5.6	183.90	30.90
<i>GCK</i> -MODY clinical criteria [2]	<45	<27			5.5–8.0 ^e	<4.5				
Number of <i>GCK</i> -MODY patients fitting clinical criteria	1/5	2/5			5/5	3/5				

Data presented as mean±SD or median (and interquartile range)

^a White Europid/Afro-Caribbean/Indian Asian/Other

^b Female unless stated otherwise

^c No valid test available – too few in some cells for χ^2 test, too many overall for Fisher's exact test

^d Alias codes for the subject and their amino acid substitutions are given

^e Range

F, female; M, male

directions identified five heterozygous mutations in five individuals (c.401 T>C, p.L134P; c.556C>T, p.R186X; c.524 G>C, p.G175A; c.1105 C>T, p.R369C; c.1364 T>A, p.V455E) and 13 non-pathogenic variants (ESM Table 2). In nine samples it was not possible to identify a base change to explain the abnormal chromatogram, giving a DHPLC screening platform a false-positive rate of 4%. The identified mutations were not identified in >400 normal chromosomes and are all at codons that are conserved across species. Two of these mutations (L134P, R186X) have previously been reported, while the remaining three novel mutations are at codons which have been mutated previously (G175A, R369C, V455E) in patients with either GCK-MODY or persistent hyperinsulinaemic hypoglycaemia of infancy because of a mutation in *GCK* [5]. Direct sequencing established that no mutations in exon 3 had been missed by the DHPLC platform.

The clinical characteristics of the 0.76% (95% CI 0.25–1.78%) participants with *GCK* mutations are consistent with GCK-MODY, but as a group they were indistinguishable from participants without *GCK* mutations (Table 1), including in terms of HOMA-derived measures of beta cell function and insulin sensitivity (ESM Fig. 1) [6]. Forty-three of 658 (6.5%) participants met all four GCK-MODY screening criteria (FPG >5.5 mmol/l; 2HPGI <4.5 mmol/l; age <45 years and BMI <27 kg/m²) in common use [2] (albeit without information on family history of diabetes), but none of these had *GCK* mutations.

Rigid application of the clinical criteria for GCK-MODY [2] in 658 overweight adults with fasting hyperglycaemia did not identify any of the 0.76% with *GCK* mutations, suggesting that many such individuals would not be identified by screening programmes using these criteria. Our results are in contrast to studies performed in children and adolescents with asymptomatic hyperglycaemia in which *GCK* mutations have been identified in 43% (35/82) of individuals [7]. Children and adolescents with hyperglycaemia are more likely to have a monogenic explanation for their condition than are older individuals (>35 years), who are more likely to be on a trajectory for type 2 diabetes. Selecting participants based on age <45 years and BMI <27 kg/m² would have missed all of those with GCK-

MODY. Our data support earlier work suggesting that GCK-MODY is a common cause of childhood and adolescent hyperglycaemia and that early screening (<25 years) would facilitate the identification of such individuals [7, 8]. In the age group we studied, the low GCK-MODY prevalence (<1%) is unlikely to affect the design and statistical power of diabetes intervention trials.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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